Molecular Biology

A PROPOSED MECHANISM FOR DEGRADATION OF TOPO II MRNA

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Determining the mechanism of degradation of mRNA is important for understanding transcriptional regulation. Topoisomerase II (topo II) is an enzyme whose levels may be regulated by its mRNA stability. Etoposide resistant K562 and K/VP.5 cells have about two times less topo II mRNA than parental K562 cells. This is due to the decrease in stability of the topo II mRNA in K/VP.5 cells and is directly related to the more rapid degradation of the topo II mRNA. The purpose of this study is to better understand the degradation process for topo II mRNA.

mRNA maybe degraded processively from the 3'-end, processively from the 5'-end or randomly degraded. To determine the most likely mechanism for topoisomerase II mRNA degradation, semi-quantitative procedures for reverse transcription (RT) and polymerase chain reaction (PCR) were executed, to determine indirectly the relative concentration of 3'-ends, 5'-ends, and a selected middle region of topo II mRNA for K562 and K/VP.5 cells. The preliminary results of this study suggest that the 3'-end topo II mRNA of K/VP.5 cells is present in a lesser concentration than for the 3'-end topo II mRNA of the K562 cells (n=3). As for the 5'-ends the concentrations are comparable for both K562 and KV/P.5 topo II mRNA (n=3). The concentration of topo II mRNA (nucleotides 2250-2541) appears to be less for K562 mRNA than for KV/P.5 mRNA (n=1). Collectively the preliminary data seem to support a model for processive degradation of topo II mRNA from the 3'-end.

Experimental plans to further examine the half-life of the 3'-end and the middle region of topo II mRNA will also be presented.